How could this frog save your life?

Antimicrobial Peptides

We all know antibiotic resistance is an increasing problem

One option for addressing this is to study naturally occurring antibiotics

Antimicrobial peptides

- Part of innate immunity of all living organisms
- kill multi-drug resistant microorganisms

Antimicrobial Peptides

What are AMPs?

Where are they made?

How do they work?

What determines their specificity?

How are they produced?

How is variety generated?

How do microbes protect themselves?

What are antimicrobial peptides?

- AMPs a.k.a. Host Defence Peptides HDPs
- Part of the innate immune system
- Found in all kingdoms of life, from bacteria to plants and animals
- Potent broad spectrum antimicrobials
- Protective by:
  - Direct toxicity to microbes
  - Activation of cells involved in inflammatory response

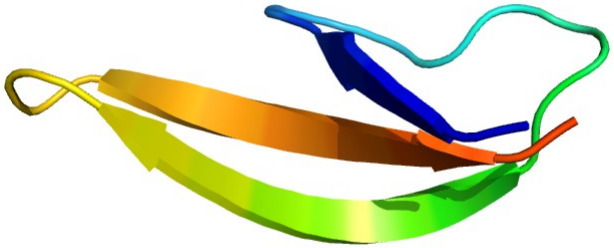
Amino acid composition of AMPs

- Small, <100 amino acids
- Net positive charge by incorporating:
  - Arg and Lys (or His in acidic environments)
- Large proportion (generally >50%) of hydrophobic residues
- Classified based on structure, amino acid composition and number of disulfide bonds

What do AMPs look like?

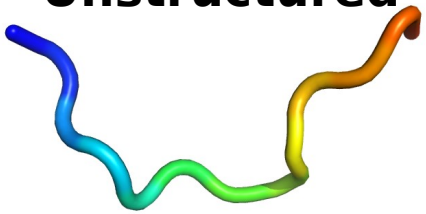
- Two main families are: defensins and cathelicidins

Defensins
β-strands with 3 disulfide bridges



Cathelicidin (LL-37)
α-helical

“Unstructured”



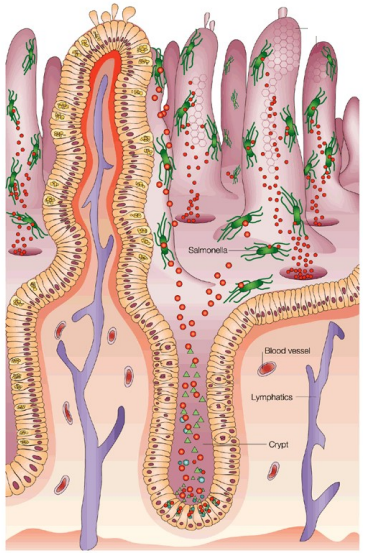
What do AMPs look like?

- Of course this is a simplification!

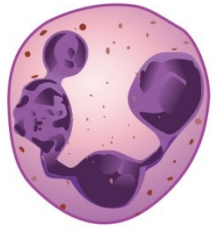
Fjell, et. al *Nature Reviews: Drug Discovery* 2012

What cell types produce AMPs?

- Epithelial cells of mucosal surfaces
  - Skin, gastrointestinal mucosa, respiratory mucosal cells



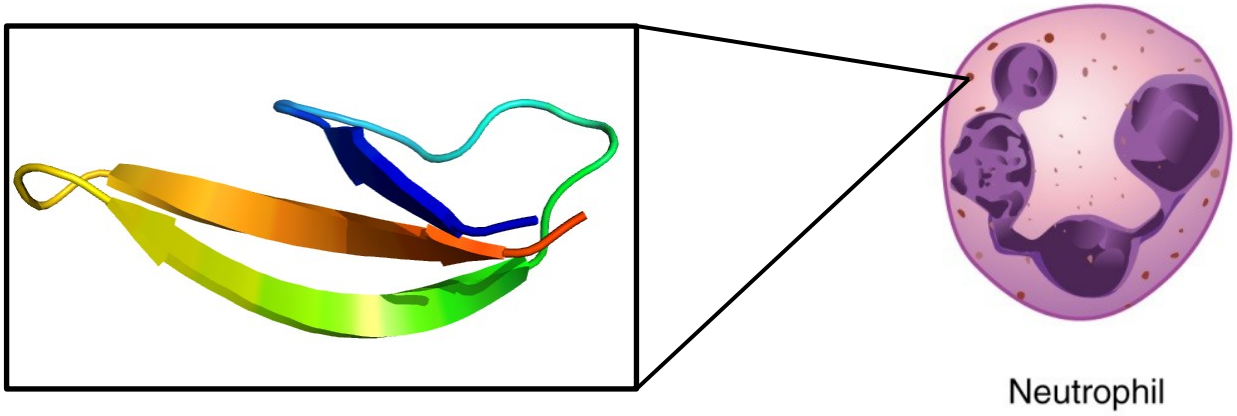
- Granule-containing leukocytes
  - neutrophils, NK cells, cytotoxic T lymphocytes



Neutrophil

High concentrations of AMPs

- Defensins:
  - can be up to mM concentrations
  - Compose 5% of total protein in neutrophils

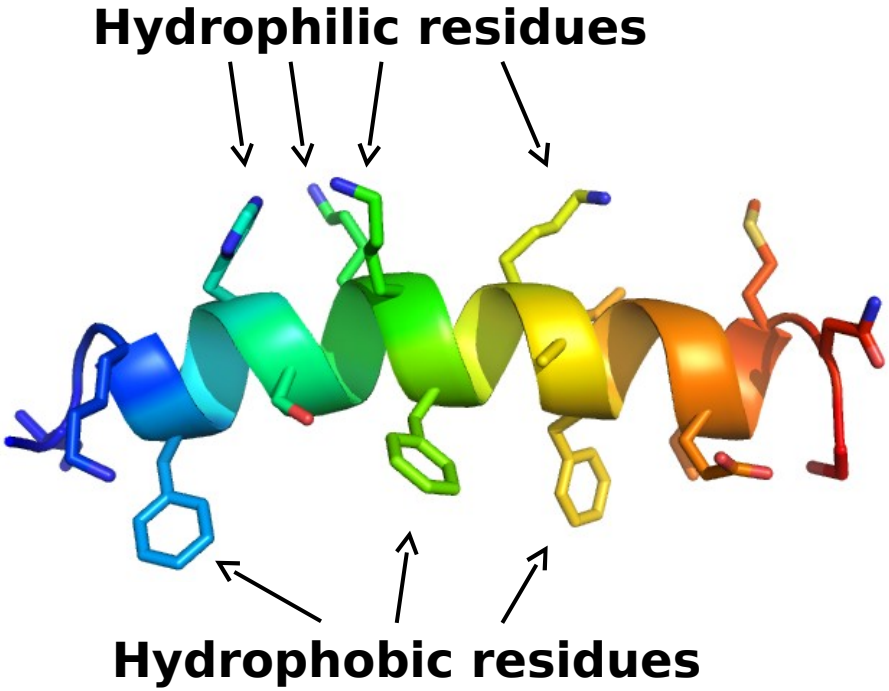


How do they work?

- Most work is focussed on their role in directly killing microbes
- Primary mode of action is thought to be membrane permeabilisation

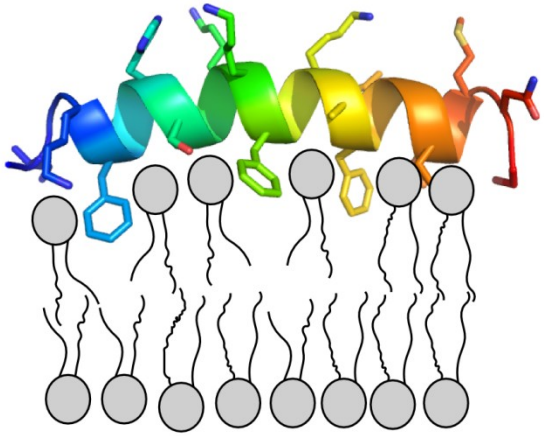
Membrane permeabilisation

- Most AMPs are amphipathic:
  - possess hydrophilic amino acids along one side and hydrophobic amino acid along the opposite side

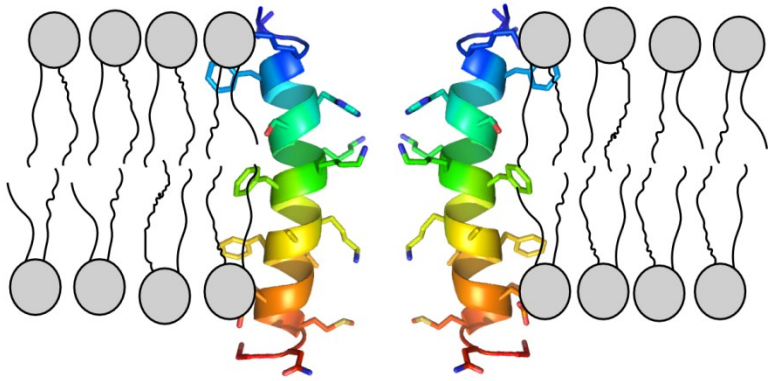


Membrane permeabilisation

- Hydrophobic sequences allow:
  - association with the surface of membrane bilayers, thus destabilising them by compromising structural integrity
  - membrane integration and formation of aqueous channels which traverse the



Carpet model



Transmembrane pore model

Membrane permeabilisation - lethality

- Leakage of small ions (H^+, Na^+, K^+) could dissipate transmembrane potential leading to depletion of ATP
- Increase in water permeability could lead to osmotic lysis
- Destroy membrane structure releasing metabolites and proteins

Other mechanisms of microbial killing

- There is some evidence that AMPs can be translocated across bilayers and into cells without causing lysis
- In this case microbial killing may involve:
  - interfering with metabolism
  - inhibition of cell wall synthesis
  - inhibition of DNA, RNA, and protein synthesis
  - inhibition of certain enzymes

Reviewed in Brogden *Nature Reviews Microbiology* 2005
Kobayashi et al *Biochemistry* 2000

What determines AMP specificity?

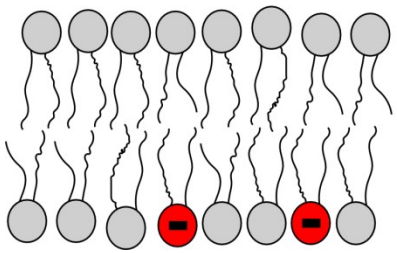
- AMPs kill
  - Gram-positive and Gram-negative bacteria
  - Mycobacteria
  - Enveloped viruses
  - Fungi (incl. yeasts)
  - Parasites (incl. Protozoa, nematodes)
  - Transformed or cancerous cells

- Why don’t they kill healthy human cells?

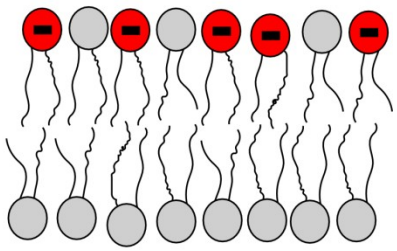
What determines AMP specificity?

- Initial contact between AMPs and target organism is electrostatic
  - Most bacterial surfaces are more anionic (-ve) than mammalian cells, contain acidic phospholipids
  - The cationic (+) properties of AMPs conferred by the [illegible] tribute most to spe

Strong electrostatic attraction



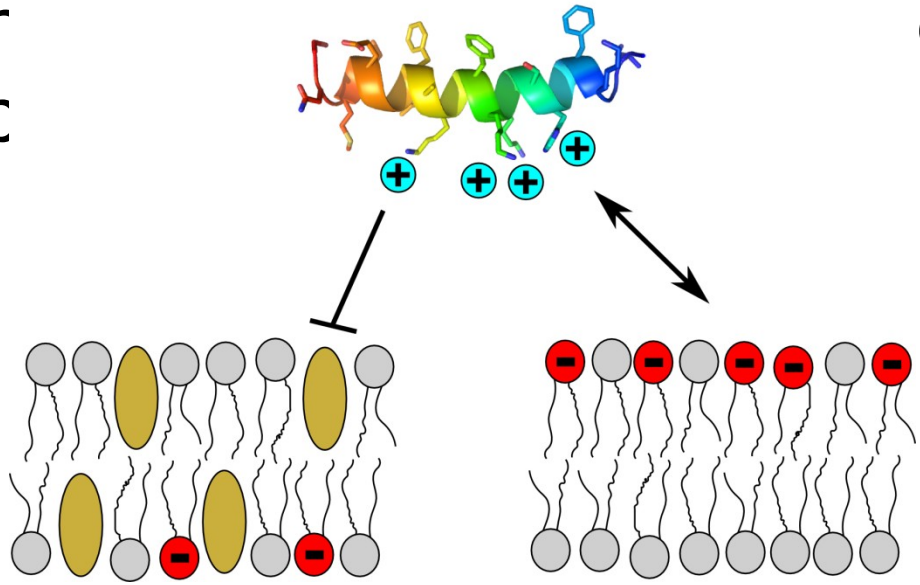
Mammalian membrane



Bacterial membrane

What determines AMP specificity?

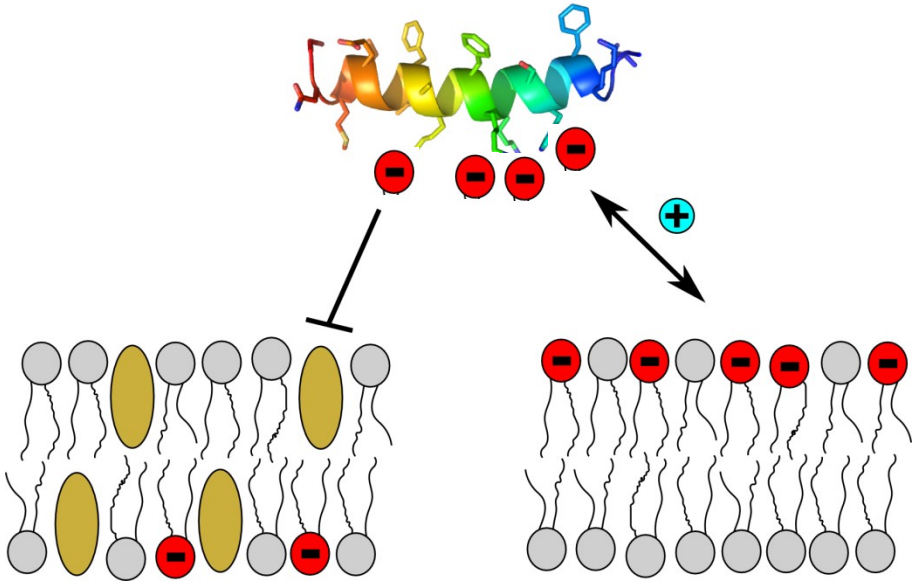
- Cholesterol widely distributed in mammalian cell membranes but absent in bacterial cell membranes
- Cholesterols reduces activity of AMPs due either to stabilization of the lipid bilayer or to inter erol and the pep



Anionic antimicrobial peptides

- Of course, it’s not quite that simple…
- Anionic AMPs also exist

  - rich in glutamate and aspartate
- But these seem to require cations such as Zn^{2+} to mediate the interaction



Other roles of AMPs: immunomodulation

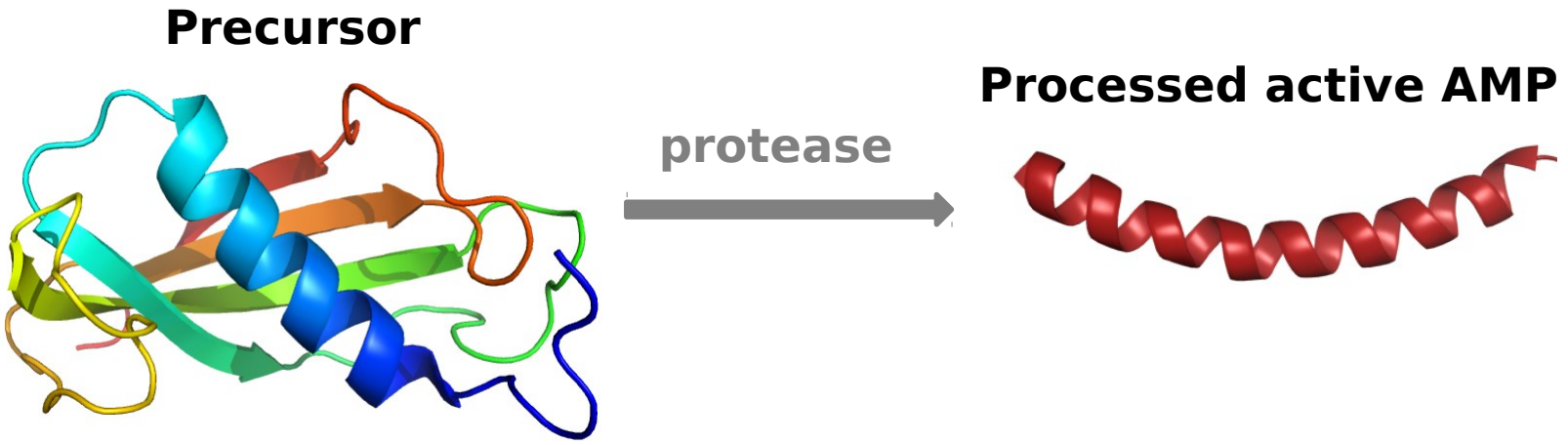
- Link between innate and adaptive immunity
- Recruit neutrophils, monocytes, mast cells and T helper cells
- Stimulate chemokine release from these cells to recruit the adaptive immune system to site of infection
- Stimulate degranulation of mast cells, promote phagocytosis, induce release of nitric oxide

How are AMPs produced?

- Depending on cell and type of AMP, secretion can be constitutive or triggered
  - Dermicidin constitutively secreted by sweat glands
  - Human β-defensins primarily triggered by inflammation
- Secretion can be enhanced by cytokines or microbial products
- AMPs are stored in secretory granules and released at mucosal surfaces or sites of infection

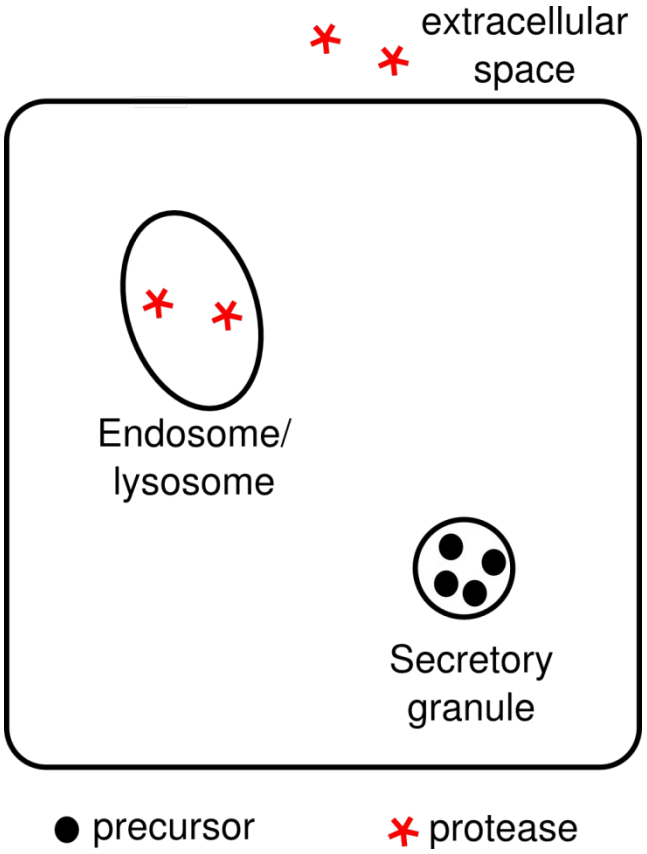
How are AMPs produced?

- AMPs are often produced as inactive precursor proteins that need to be cleaved by proteases for activity



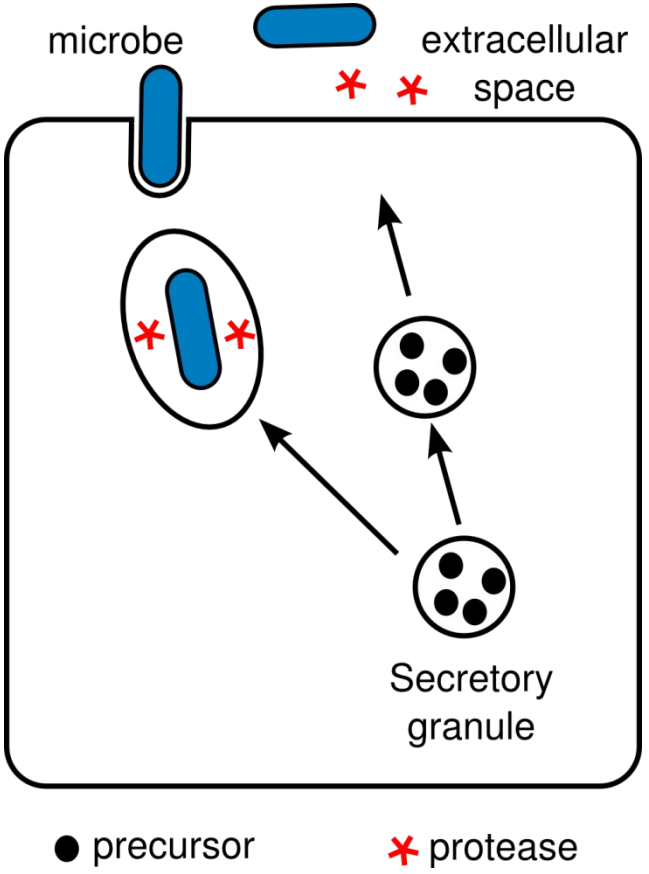
How is proteolysis controlled?

- Proteases and precursor proteins are kept separate
- The inactive precursor is often stored in granules
- The proteases are often extracellular or in lysosomal compartments



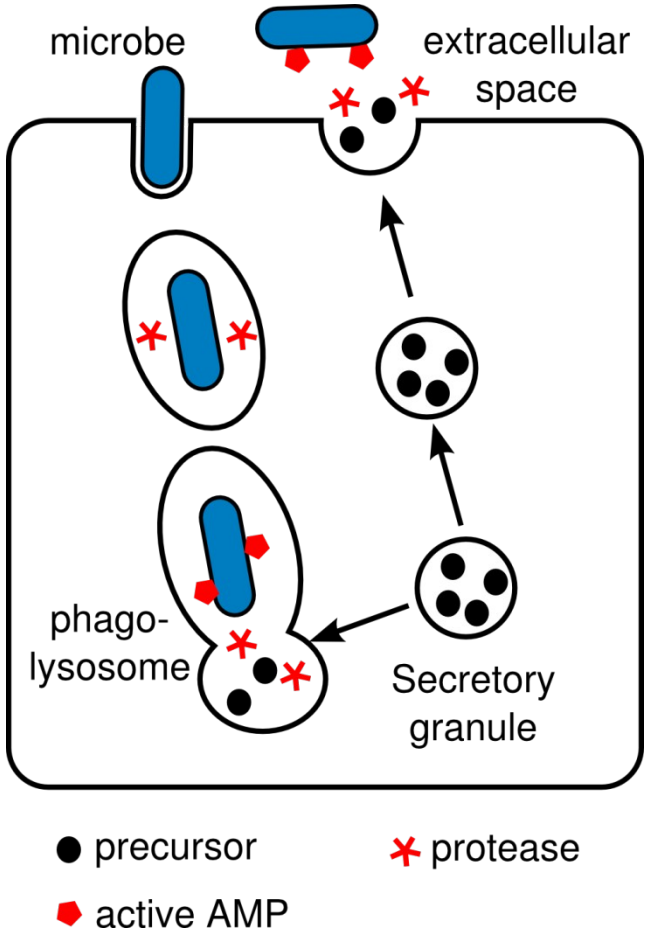
How is proteolysis controlled?

- When a pathogen is detected, granules are mobilised and fuse with the plasma membrane to be secreted or with the phagocytic vesicle



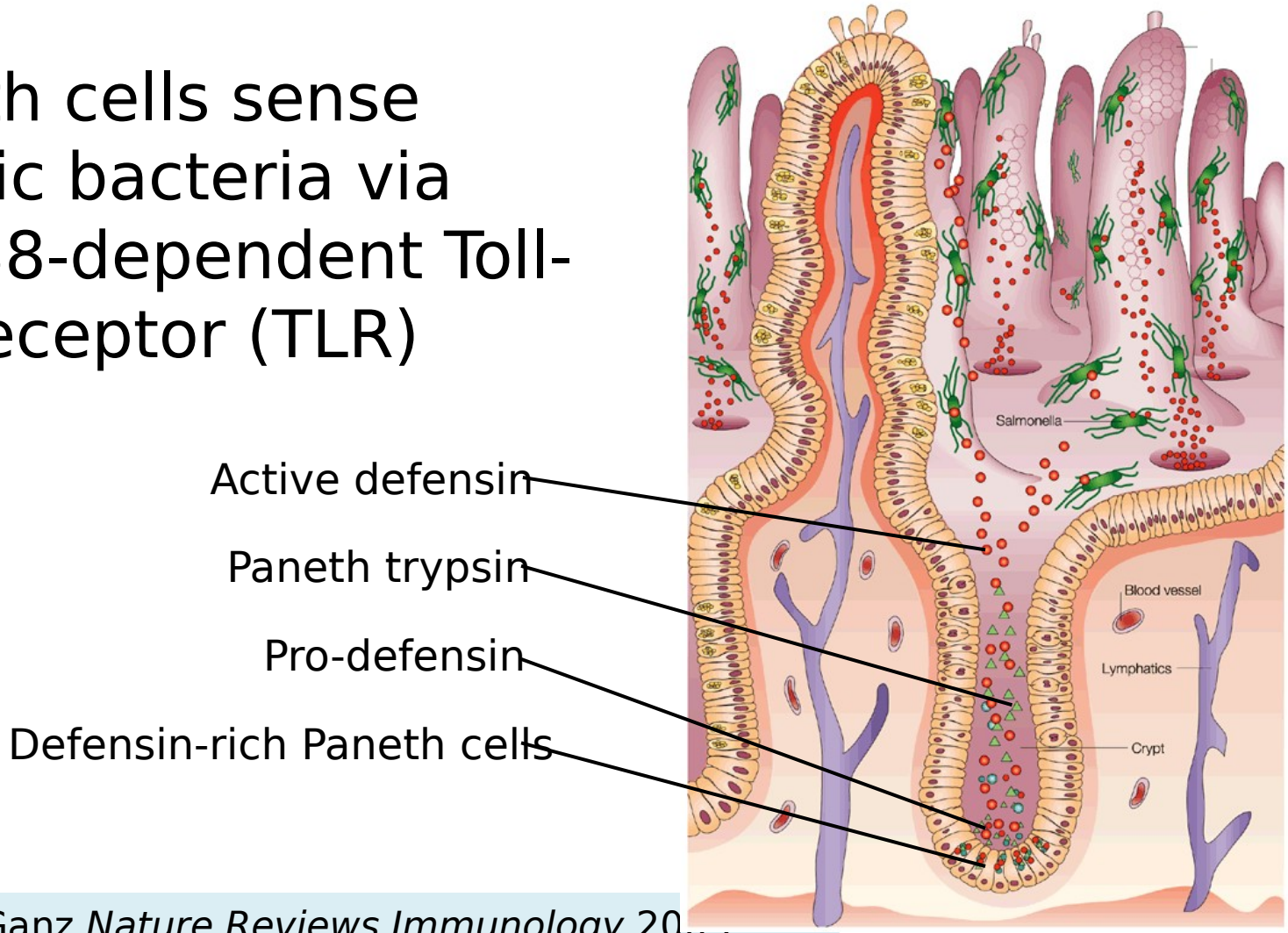
How is proteolysis controlled?

- The precursor proteins are then cleaved by the relevant protease to produce the active AMPs



Killing of Salmonella by human defensins

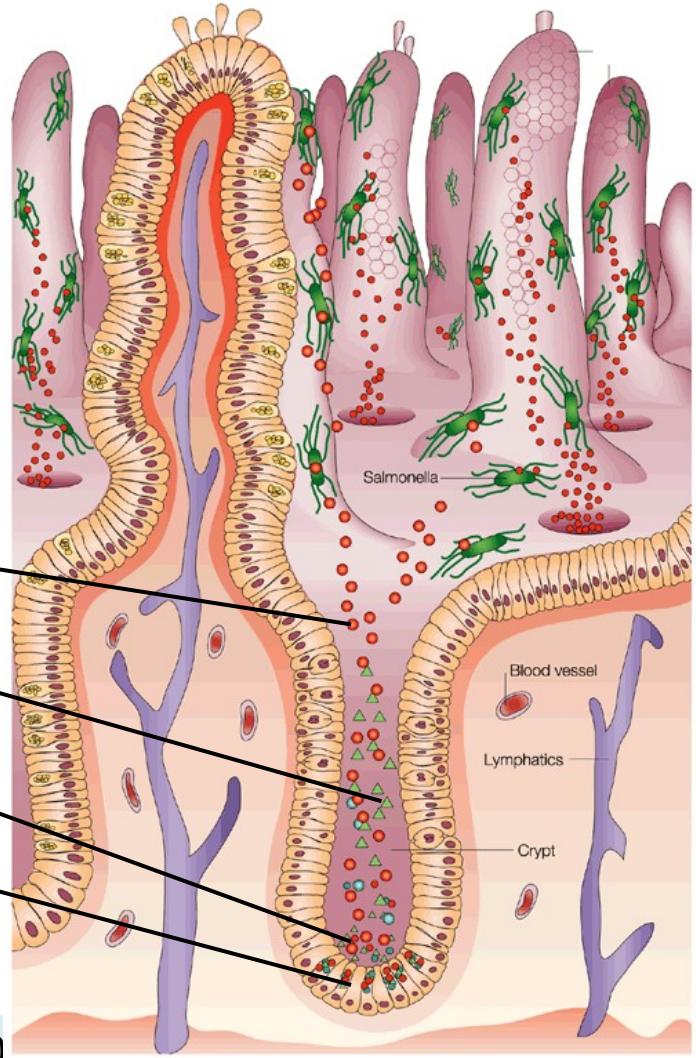
- Paneth cells sense enteric bacteria via MyD88-dependent Toll-like receptor (TLR)



Reviewed in Ganz *Nature Reviews Immunology* 20
Original papers: Salzman et al *Nature* 2003, Vaishnava et al PNAS 2008

Killing of Salmonella by human defensins

- Restricting activation to the intestinal lumen might protect the epithelium and crypt (with intestinal stem cells)

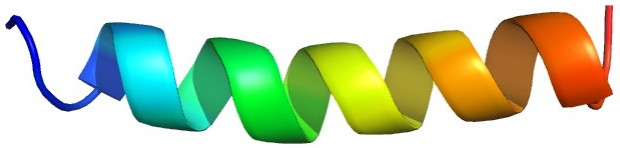


Reviewed in Ganz *Nature Reviews Immunology* 20
Original papers: Salzman et al *Nature* 2003, Vaishnava et al PNAS 2008

Example: Cathelicidin hCAP-18

- The only human cathelicidin is hCAP-18
- Major protein of specific granules of neutrophils
- hCAP-18 is an AMP precursor
- Processed to the AMP LL-37 by lysosomal proteinase 3

hCAP-18 **proteinase -3** →



Sorensen, et. al *Blood* 2001

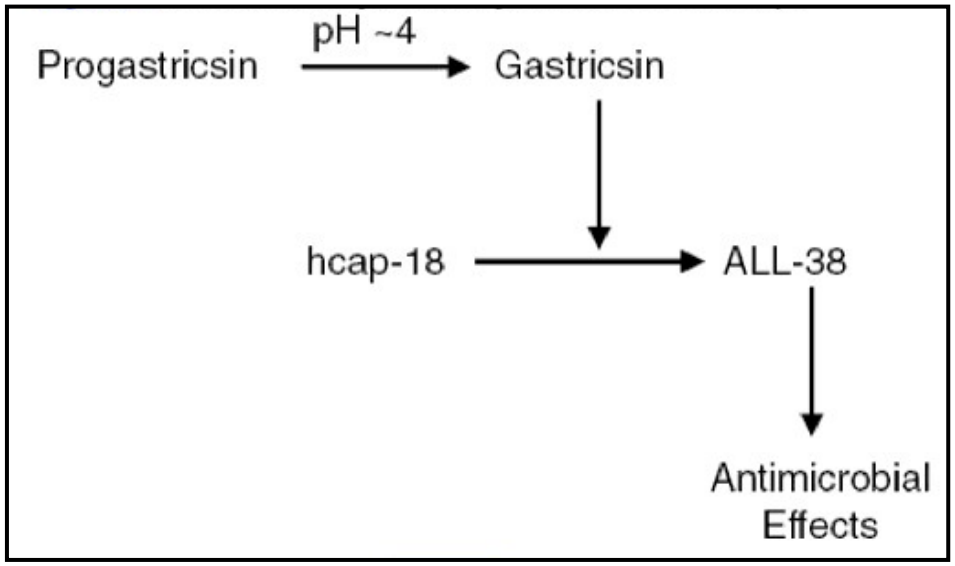
Example: Cathelicidin hCAP-18

- But it is also present in its unprocessed, inactive form in seminal plasma
- Prostate-derived protease, gastricsin, is also in seminal fluid but inactive at this basic pH
- In the acidic environment of the vagina gastricsin is activated and hCAP-18 is processed to the AMP ALL-38
- ALL-38 has antimicrobial activity against a variety of micro-organisms

Sorensen, et. al *J Biol. Chem.* 2003

Example: Cathelicidin hCAP-18

- As gastricsin remains inactive in the seminal fluid
- And is only activated in the low pH environment of the vagina
- This is a novel mechanism to prevent infection following sex



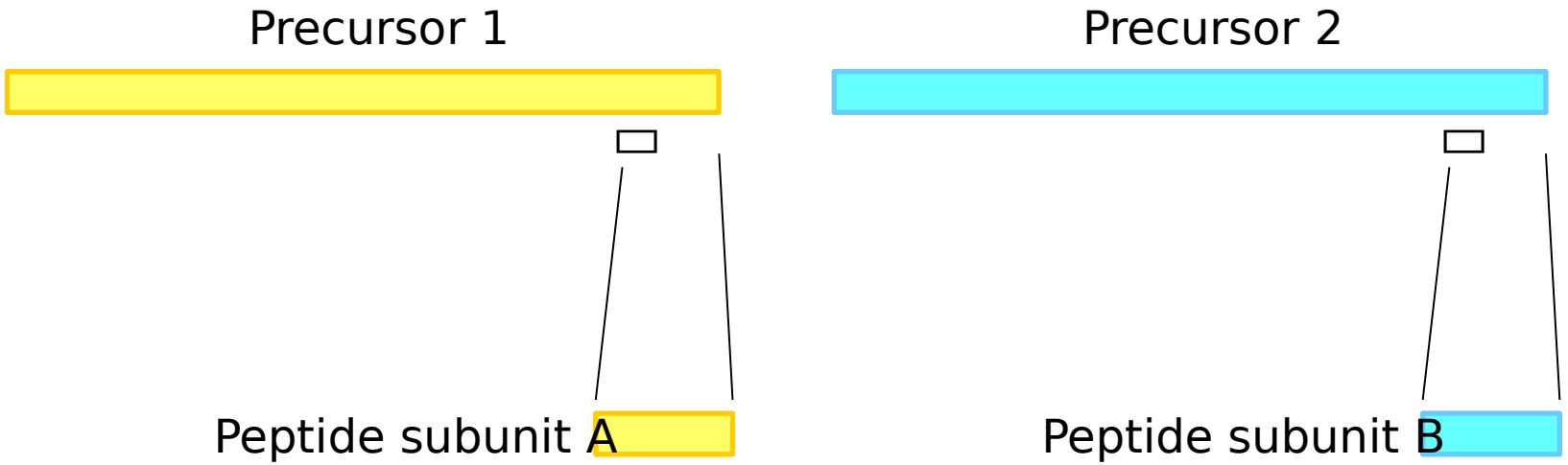
Sorensen, et. al *J Biol. Chem.* 2003

How is variety in AMPs generated?

- Variety generated at the protein level
- As just described, the same precursor can be cleaved by different proteases
  - Human hCAP-18 by proteinase-3 or gastricsin
  - Furthermore, the proteases used by related species differ. Bovine and porcine cathelicidins are cleaved by elastase
- Short peptides can be fused and cyclised in different combinations

Theta defensins

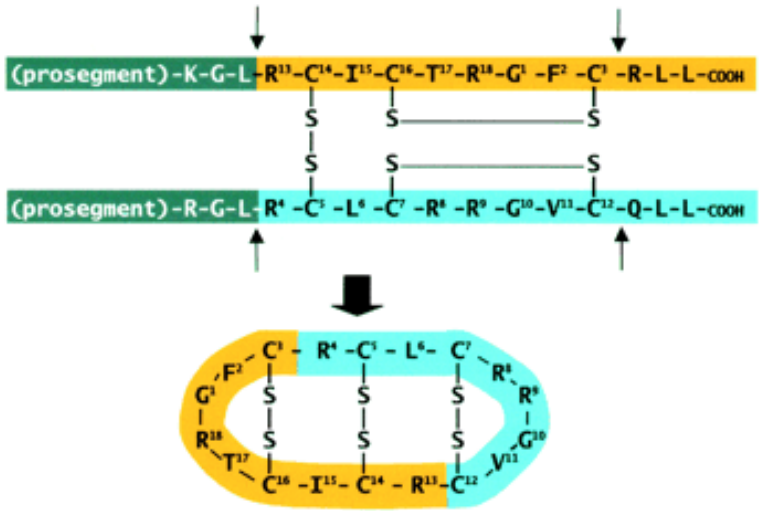
- Theta-defensins: cyclic peptides found in some non-human primates
- 4 precursor proteins of 76 residues each are processed into 9-residue peptides: subunits A,B,C,D

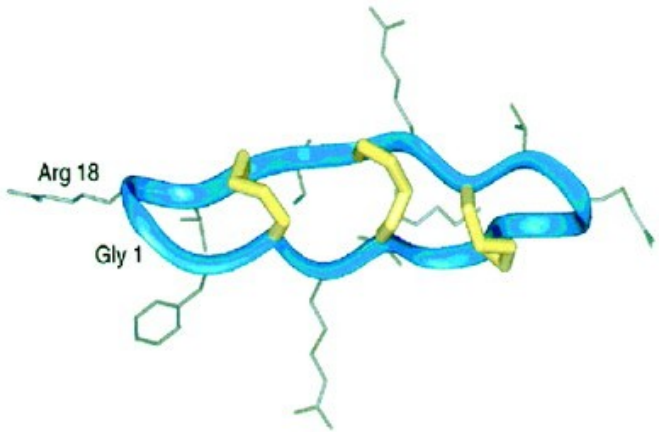


Tang, et. al *Science* 1999

Theta defensins

- 2 of these peptides are spliced together to form a 18 residue circular defensin with 3 disulfides





Tang, et. al *Science* 199

Theta defensins

- These four subunits could combine to produce 10 different defensins. So far five have been found:
  - TD-1: A and a B subunit
  - TD-2: two B subunits
  - TD-3: two A subunits
  - TD-4: A and a C subunit
  - TD-7: A and a D subunit
- Protect macaques, baboons and orangutans from HIV-like viruses

Tang, et. al *Science* 1999

“Human θ-defensin” or retrocyclin

- Human genome contains theta-defensin genes, but they have a premature stop codon, so are non-functional
- An artificial human theta-defensin, retrocyclin, was created by `fixing' the pseudogene
- *In vitro* it has been shown to be effective against HIV, herpes simplex virus and influenza A
- Acts primarily by preventing these viruses from entering their ta

Venkataraman, et. al *PLoS Biology* 2009
Reviewed in Penberthy, et. al *Cell Mol Life Sci* 2011

“Human θ-defensin” or retrocyclin

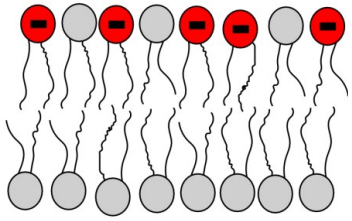
- Many questions remain to be answered
  - The mRNA is still produced but cannot be translated into a functional defensin
    - Why is the remainder of the gene conserved?
    - Why is the machinery conserved for making cyclic defensins?
  - When in evolution did the stop codon mutation appear?
  - Is there an alternative role for this gene product?

Reviewed in Penberthy, et. al *Cell Mol Life Sci*, 2011

How do bacteria defend themselves?

1. Modify their cell surface

- Alteration of surface charge to repel AMP binding
  - Modify anionic surface molecules including peptidoglycan, teichoic acid, lipopolysaccharide and membrane phospholipids

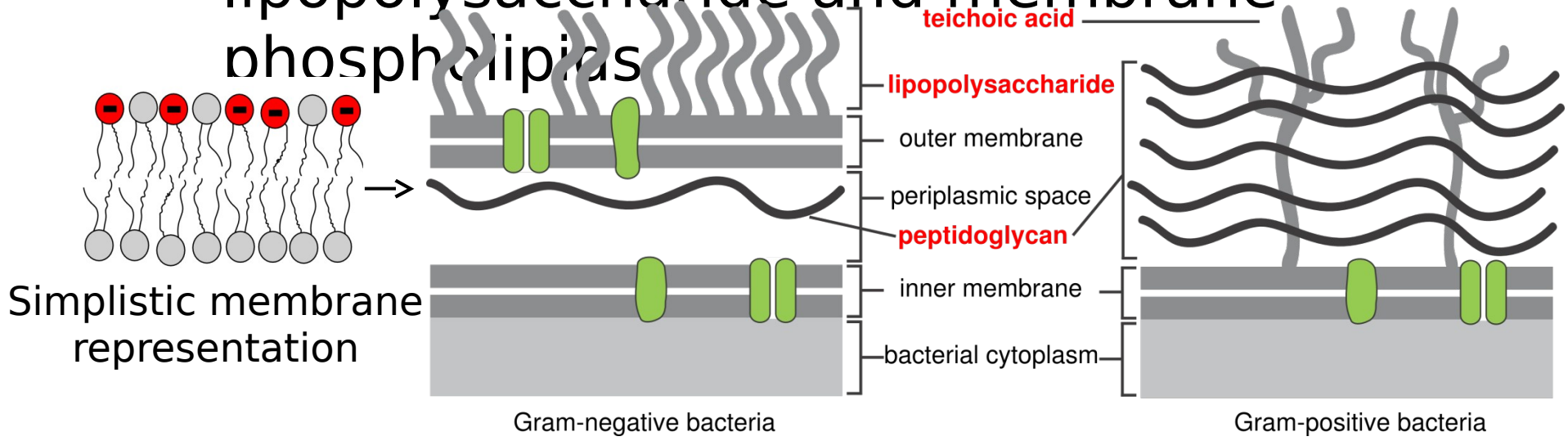


Simplistic membrane representation

How do bacteria defend themselves?

1. Modify their cell surface

- Alteration of surface charge to repel AMP binding
  - Modify anionic surface molecules including peptidoglycan, teichoic acid, lipopolysaccharide and membrane phospholipids



How do bacteria defend themselves?

1. Modify their cell surface

- Alteration of surface charge to repel AMP binding
  - Modify anionic surface molecules including peptidoglycan, teichuronic acid, lipopolysaccharide and membrane phospholipids
- Alter bacterial membrane fluidity to reduce membrane permeability

Reviewed in Koprivnjak, et. al *Cell Mol. Life Sci.* 2011

How do bacteria defend themselves?

2. Inactivate and cleave AMPs by producing proteases and binding proteins

- Binding proteins sequester AMPs reducing their activity against the pathogen
- Secreted and cell-envelope anchored proteases are common virulence factors in bacterial pathogens
- Possible that this evolutionary pressure led to the incorporation of disulfide bridges making AMPs more resistant to proteolysis

Reviewed in Koprivnjak, et. al *Cell Mol. Life Sci.* 2011

How do bacteria defend themselves?

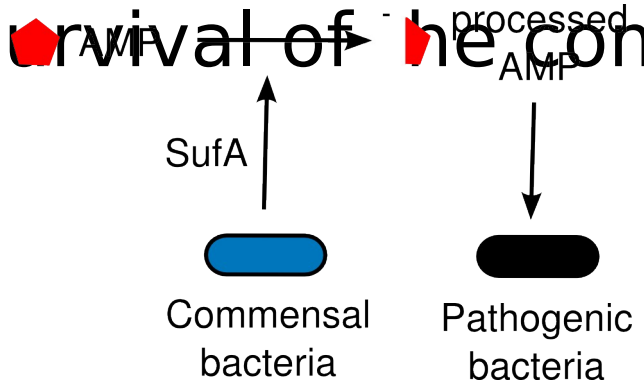
3. Expulsion of AMPs using multi-drug efflux pumps

- AMPs that damage the membrane and end up in the bacterial cytoplasm can be exported by multi-drug efflux pumps
- These can expel diverse antibiotics and confer resistance to AMPs
- However, there is conflicting data regarding their exact role

Reviewed in Koprivnjak, et. al *Cell Mol. Life Sci.* 2011

Resistance by commensals

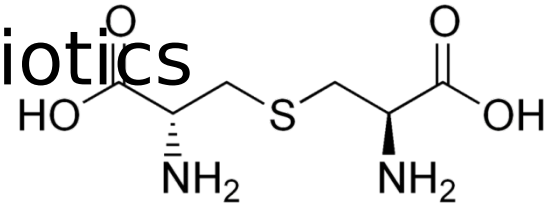
- Enzyme SufA is produced by commensal bacteria that reside on the skin (the normal microbiota)
- The products of AMP degradation by SufA are potent against pathogenic bacteria promoting survival of the commensal



Frick, et. al *J.Immunol.* 2011

Bacterial production of AMPs

- Bacteria produce their own AMPs to reduce competition from other bacterial strains
- Some are broad spectrum, some narrow spectrum
- Known as bacteriocins and include lantibiotics



Lanthionine

Special amino acid not found in proteins (so also not cleaved by normal proteases)

Bacterial production of AMPs

- Examples of bacterial AMPs include:

  - Colicin produced by *E. coli* strains to attack other *E.coli* strains

  - Vibriocins from *Vibrio* sp. active against Gram –ve bacteria

  - Nisin from *Lactococcus* sp. which incorporates uncommon amino acids (e.g. lanthionine) and is used in food preservation

AMP-related Diseases

- Dysregulation of the generation of AMPs plays a role in several human diseases
- Deficiencies result in severe and frequent infections
- AMPs may also play a role in:
  - Some cancers, such as colorectal cancer
- Excessive AMP production may play a role in several inflammatory conditions including:
  - Arthritis
  - Atherosclerosis
  - Crohn's disease

AMP-related Diseases

- Dysregulation of the generation of AMPs plays a role in several human diseases
- Deficiencies result in severe and frequent infections
- AMPs may also play a role in:
  - Some cancers, such as colorectal cancer
- Excessive AMP production may play a role in several inflammatory conditions including:
  - Arthritis
  - Atherosclerosis
  - Crohn's disease – gut microbiota vs. host AMPs

Review: Ostaff, et. al *EMBO Mol. Med.* 2013

Membrane-active peptides

- Are AMPs the whole story?

  - There are other short amphiphilic peptides that interact with membranes

- Pre-amyloid toxins (PAT)
- Cell-penetrating peptides (CPP)

Review: Last, et. al *Protein Sci.* 2013

Membrane-active peptides

- Are AMPs the whole story?

  - There are other short amphiphilic peptides that interact with membranes

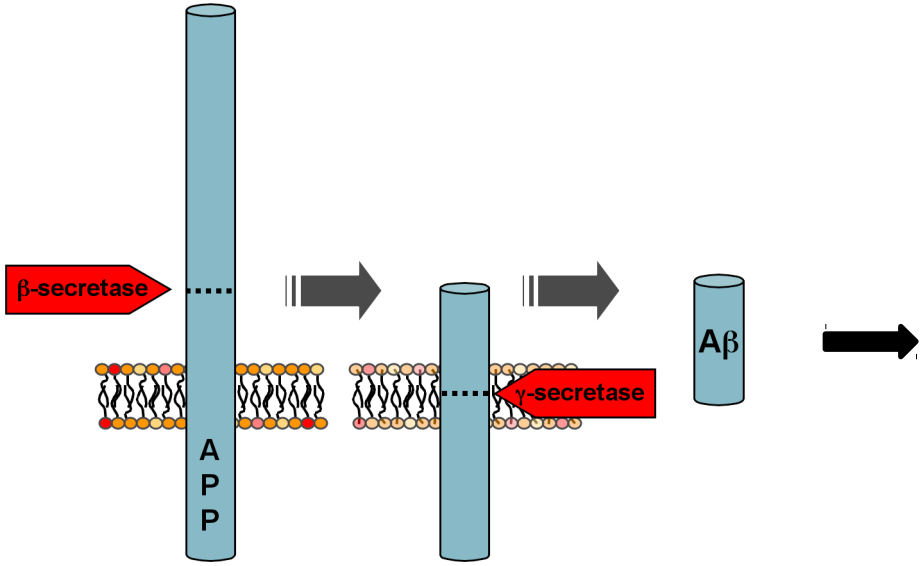
- Pre-amyloid toxins (PAT)
- Cell-penetrating peptides (CPP)

- Are these classes distinct? There is some evidence that they are not

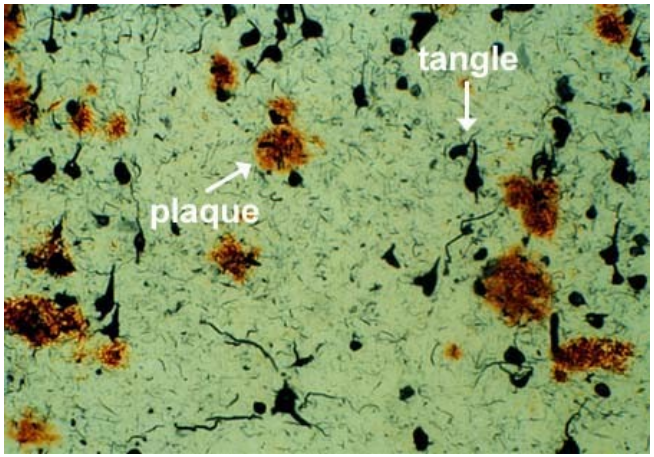
Review: Last, et. al *Protein Sci.* 2013

Pre-amyloid toxins as AMPs

- Is the Aβ peptide that is found in plaques in Alzheimer's patients an AMP?

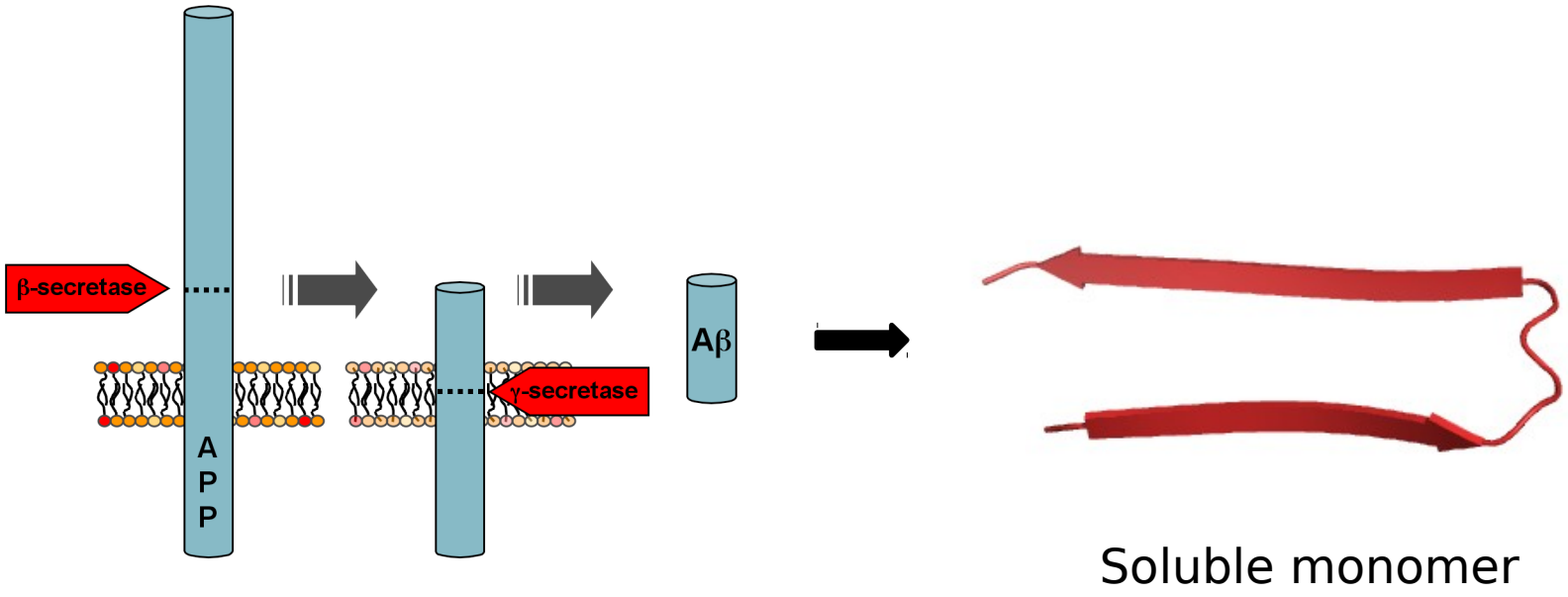


Amyloid Precursor Protein (APP)



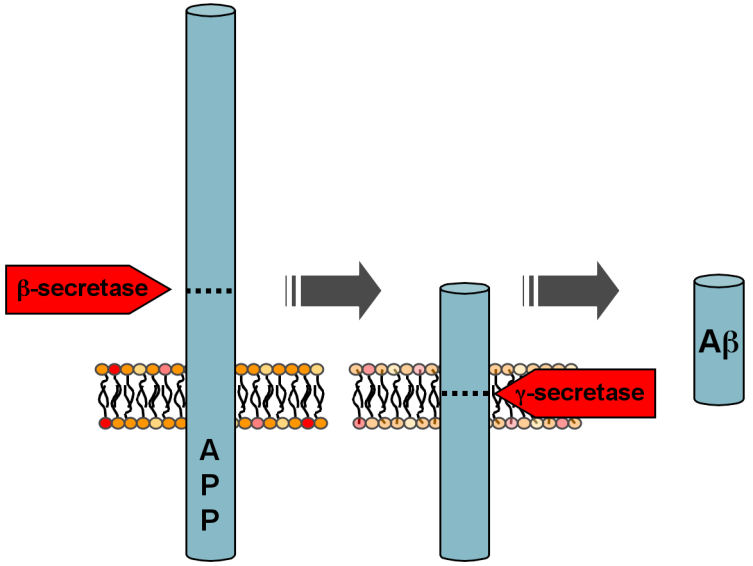
Pre-amyloid toxins as AMPs

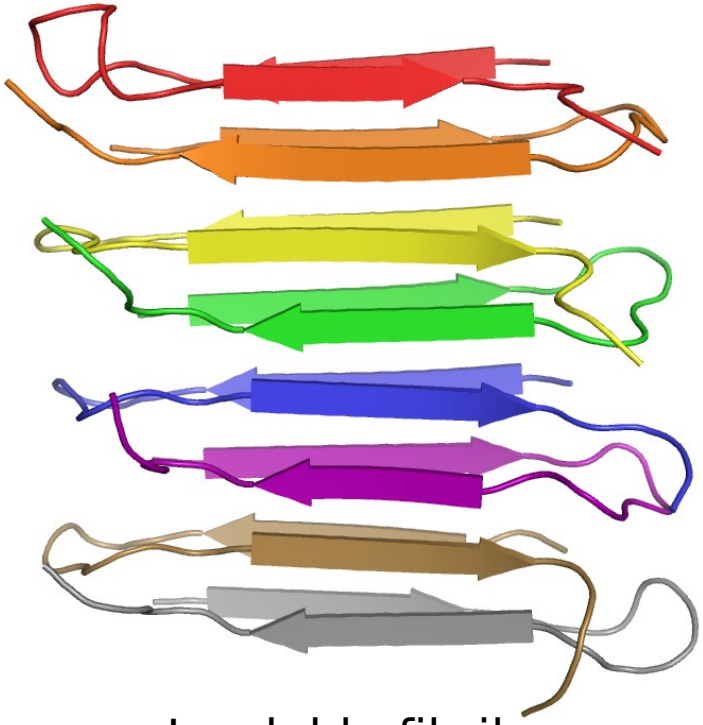
- Is the Aβ peptide that is found in plaques in Alzheimer's patients an AMP?



Pre-amyloid toxins as AMPs

- Is the Aβ peptide that is found in plaques in Alzheimer's patients an AMP?

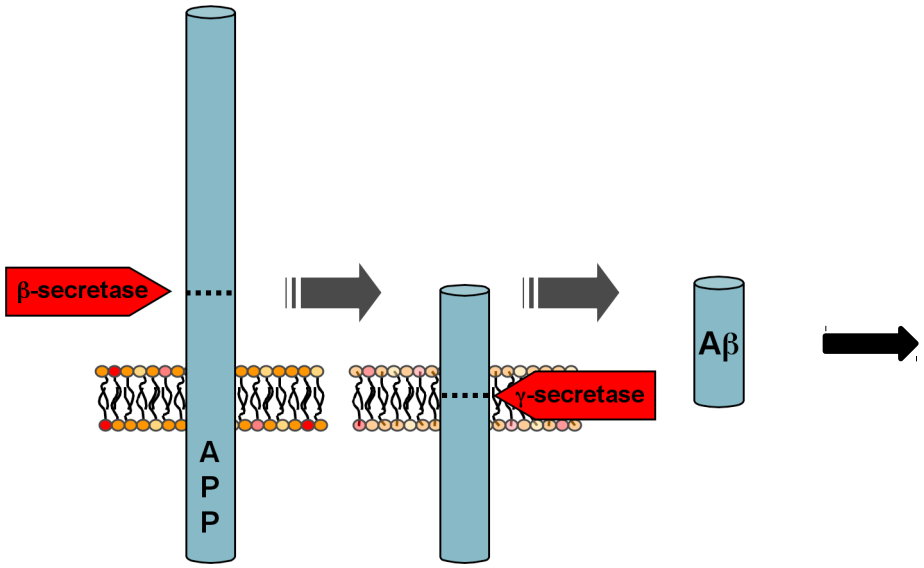




Insoluble fibrils found in plaques

Pre-amyloid toxins as AMPs

- Is the Aβ peptide that is found in plaques in Alzheimer's patients an AMP?

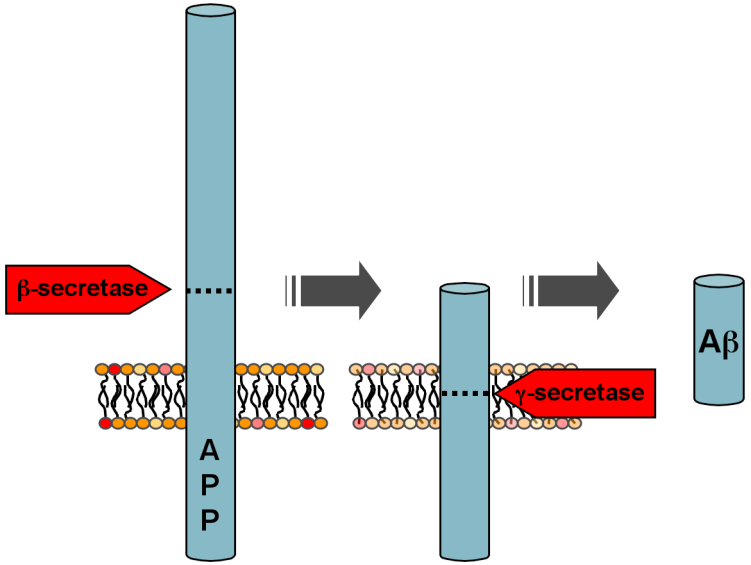


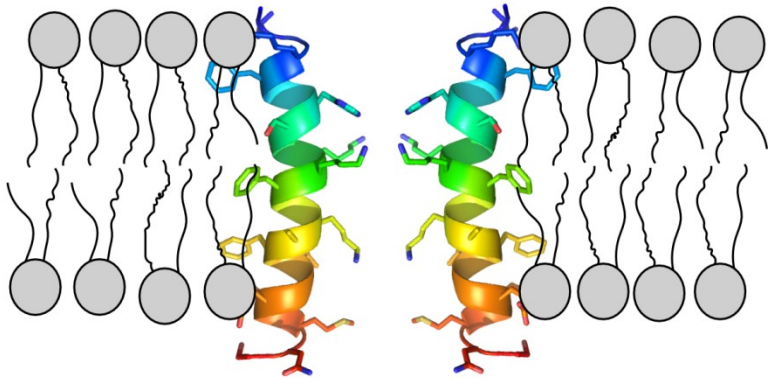
Cytotoxic, pore-forming soluble oligomer?

Laganowsky, et. al
Science 2012

Pre-amyloid toxins as AMPs

- Is the Aβ peptide that is found in plaques in Alzheimer's patients an AMP?





Cytotoxic, pore-forming soluble oligomer?

Pre-amyloid toxins as AMPs

- Recent data suggests that:
  - the naturally occurring Aβ peptide possesses antimicrobial activity with a potency equivalent to LL-37
  - Alzheimer's brain tissue has higher antimicrobial activity than age-matched controls
  - Antibodies against Aβ ablate antimicrobial activity
  - mice lacking the proteases needed to generate Aβ have increased susceptibility to microbial infections

Soscia, et. al *PLoS One* 2010
Reviewed in Harris et. al *FASEB J* 2012

Pre-amyloid toxins as AMPs

- Recent data suggests that:
  - Aβ is localised to the mitochondrial membrane
  - Mitochondrial damage is frequently evident in dementia patients

Anandatheerthavarada, et. al *J. Cell Biol.* 2003
Du et. al *PNAS* 2010

Pre-amyloid toxins as AMPs

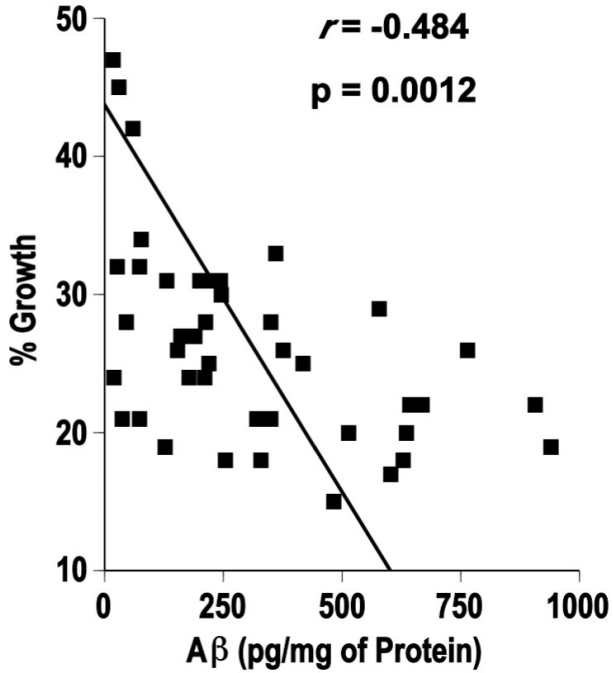
- Recent data suggests that:
  - Aβ is localised to the mitochondrial membrane
  - Mitochondrial damage is frequently evident in dementia patients

Is there a relationship between defence against microbial infections and development of neurodegenerative disorders?

Anandatheerthavarada, et. al *J. Cell Biol.* 2003
Du et. al *PNAS* 2010

Pre-amyloid toxins as AMPs

- However, it’s early days in this field and more data are required



Soscia, et. al *PLoS One* 2010

Novel therapeutics – the good

- Broad spectrum – *actually they're also anti-viral*
- Act without high specificity so reduce resistance
- Are bacteriocidal as opposed to bacteriostatic
- Require a short contact time to induce killing

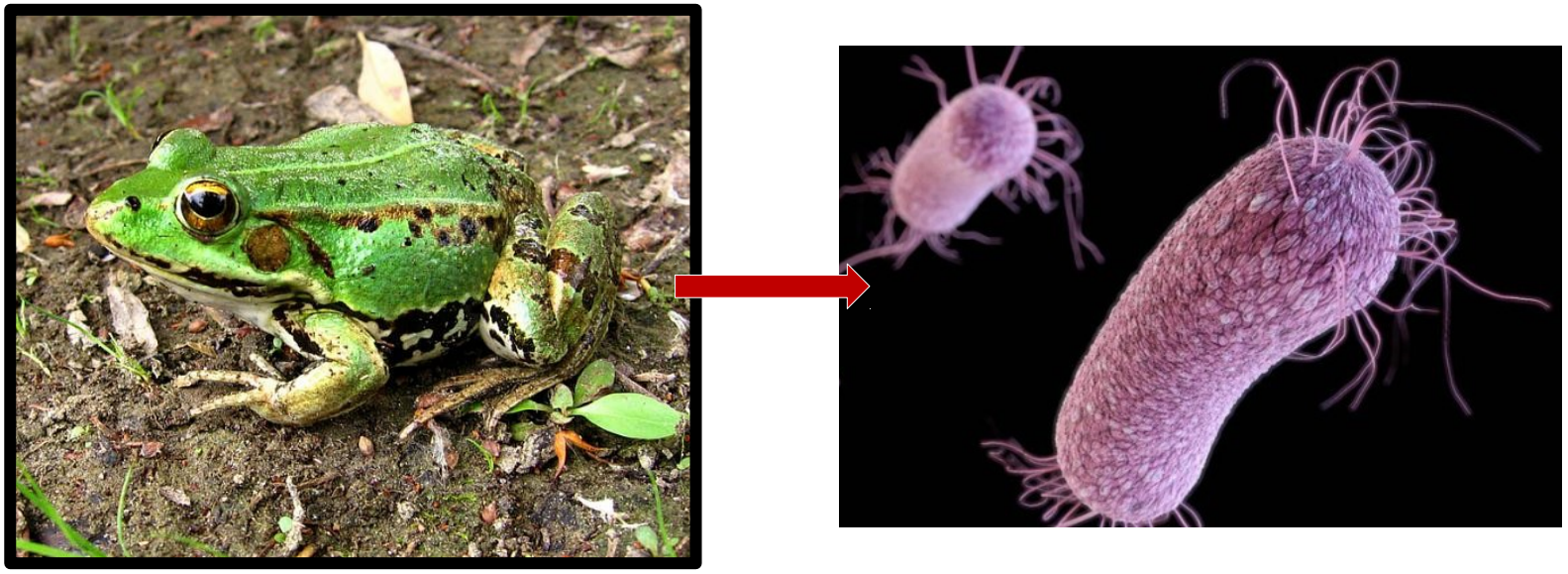
Novel therapeutics – the good

- Broad spectrum – *actually they're also anti-viral*
- Act without high specificity so reduce resistance
- Are bacteriocidal as opposed to bacteriostatic
- Require a short contact time to induce killing

- Some naturally occurring peptides have been developed as novel therapies for wound infections, lung infections associated with cystic fibrosis and cancer

Frog AMPs

Specialised peptides present in frog skin are active antibiotics against multi-drug resistant infections



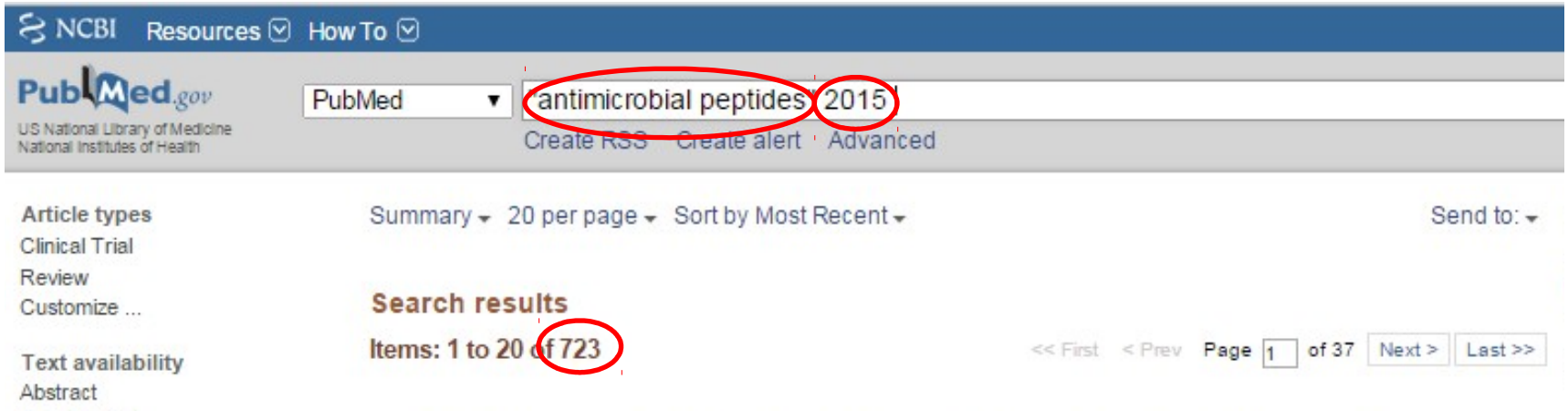
Uccelletti, et. al, *Antimicrobial Agents and Chemotherapy*, 2010

Novel therapeutics – the bad

- High concentrations are needed in order to be effective
- Potentially harmful immunomodulatory effects may make them unsuitable
- Developing resistance to AMPs potentially more dangerous than resistance to antibiotics
- Inhibitors of enzymes responsible for changing surface charge of bacteria appear to be promising

Ongoing Story

- There are still lots of unanswered questions in this field



- ...not “The End”